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Docking Interactions of Hematopoietic Tyrosine Phosphatase with MAP Kinases ERK2 and p38α

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Supporting Information

ABSTRACT: Hematopoietic tyrosine phosphatase (HePTP) regulates orthogonal MAP kinase signaling cascades by dephosphorylating both extracellular signal-regulated kinase (ERK) and p38. HePTP recognizes a docking site (D-recruitment site, DRS) on its targets using a conserved N-terminal sequence motif (D-motif). Using solution nuclear magnetic resonance spectroscopy and isothermal titration calorimetry, we compare, for the first time, the docking interactions of HePTP with ERK2 and p38α. Our results demonstrate that ERK2−HePTP interactions primarily involve the D-motif, while a contiguous region called the kinase specificity motif also plays a key role in p38α−HePTP interactions. D-Motif−DRS interactions for the two kinases, while similar overall, do show some specific differences.

In higher eukaryotes, the pleiotropic mitogen-activated protein (MAP) kinases (ERK1−5; p38α, -β, -γ, and -δ; and JNK1−3) phosphorylate specific serine/threonine residues on their target proteins, leading to cellular responses as diverse as proliferation, differentiation, survival, and apoptosis.[1] MAP kinase signaling cascades are mediated by "docking" interactions of the kinases with their substrates, phosphatases, and adapter proteins.[2] These docking interactions involve specific structural motifs on the MAP kinases and conserved sequence motifs on their interaction partners. Docking interactions with hematopoietic tyrosine phosphatase (HePTP, a class I protein tyrosine phosphatase) allow the deactivation of both extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38α by selective dephosphorylation of their activation loop tyrosines and also help to sequester the kinases in their resting state in the cytosol.[3] These interactions require a region located on the disordered N-terminus of HePTP containing a consensus $(R/K)_{2-3}$-X_{2-6}-ϕ_A-X-ϕ_B sequence (ϕ_A and ϕ_B are hydrophobic residues) known as a kinase interaction motif (KIM),[4] a DEJL motif,[5] a D-site, or a D-motif.[6] This sequence motif (we use D-motif here) is recognized by the D-recruitment site (DRS) on MAP kinases.[7,8] The MAPK DRS consists of two distinct subsites; one (Φ_{chg}) forms electrostatic and the other (Φ_{hyd}) hydrophobic interactions with D-motif sequences.[9] Crystallographic analyses largely involving short peptide ligands have revealed the versatility of D-motif−DRS interactions,[2,7,8,10] but attempts to crystallize complexes formed by full-length kinases with regulatory proteins have mostly been unsuccessful, likely because of the inherent flexibility of elements outside the canonical docking regions. Recently, we used a combination of small angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR) spectroscopy to study docking interactions involving ERK2[11] and p38α[12] utilizing peptide ligands as well as full-length HePTP. For p38α in the resting state,[12] the NMR spectral perturbations in the presence of either a 17-residue peptide encoding the D-motif (KIM) sequence of HePTP (KIM_{15-31}) or full-length HePTP revealed engagement of both Φ_{chg} and Φ_{hyd} subsites of the DRS. This contrasts with available crystallographic data for p38α in complex with short D-motif peptides that appear to indicate a pronounced engagement of only the Φ_{hyd} subsite.[10,13] Crystal structures of ERK2−D-motif peptide complexes[7,8] exhibit engagement of both DRS subsites, suggesting possible differences in D-motif−DRS interactions between ERK2 and p38α. However, engagement of the Φ_{chg} subsite was seen in a crystal structure of the complex of p38α with a substrate, full-length MAPKAP kinase 2 (MK2),[14] and also in a complex with the MAP kinase binding domain of dual-specificity phosphatase MKP5.[15] Given these contrasting results, we used solution NMR spectroscopy to compare D-motif−DRS interactions involving p38α and ERK2 in their resting states using the same peptide ligand, KIM_{15-31}, and full-length HePTP (called HePTP hereafter). Our results provide for the first time a comparison of the interactions of two MAP kinases with the same D-motif peptide and the same full-length phosphatase. We show that HePTP recognizes ERK2 mainly through the D-motif and p38α through both the D-motif and a contiguous kinase specificity (KIS)[16] motif. The catalytic PTP domain of

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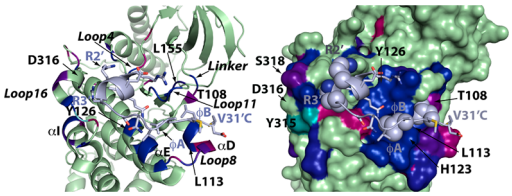

Figure 1. Close-up of spectral perturbations induced by KIM_{15-31}, plotted using a red (minimal perturbation) to blue (maximal perturbation) gradient (scale optimized for maximal visual contrast), on ribbon (left) and surface (right) representations of an ERK2 mutant bound to KIM_{16-31} with a V31′C mutation (KIM_{16-31m}) (light blue). Residues with perturbations below the 0.041 ppm threshold are colored green. Resonances that disappear upon binding are colored cyan. In the right panel, side chains belonging to the consensus D-motif sequence (^{20}R-^{21}R-X_5-^{27}L-X-^{29}L) are shown as spheres.

HePTP does not appear to impart any significant structural stability in the binding of either ERK2 or p38α.

Focusing first on the spectral perturbations (Figure 1) induced on the ERK2 DRS by KIM_{15-31}, we noted an excellent agreement with those expected from crystallographic studies (see below).[8] Several Φ_{chg} subsite residues on loop 16 (Y315, D316, and E320) and loop 4 (H78 and N80) were affected. Unexpectedly, the common-docking (CD) residue (D319 in ERK2), whose side chain was deemed critical for binding, is minimally perturbed (D316 on p38α is also minimally perturbed). This may be explained by the complex dependence of backbone spectral perturbations in the case of interactions involving side chains on the induced conformational changes affecting the residue in question as well as the neighboring ones. In principle, different contributions may cancel each other; hence, for isolated perturbations (or lack thereof), the possibility of false negatives has to be included. The fact that both residues flanking the CD Asp are perturbed suggests that this is likely the case here. Similarly, all of the structural elements comprising the Φ_{hyd} subsite (loop 8, loop 11, and helices αD and αE) were perturbed. This includes Y126 that contacts the peptide L27′ (ϕ_A, peptide residues primed) side chain and both L113 and L155, which form the binding pocket for L29′ (ϕ_B). We use the original definition[8] of the hydrophobic residues based on sequence. The side chains of the perturbed H123 (αE) and Q117 (loop 8) form hydrogen bonds with the peptide backbone in the crystal structure.

In the initial crystal structures of the ERK2−KIM_{16-31} complex, V31′, the terminal residue of KIM_{16-31}, was found to be disordered.[8] However, a V31′C mutation in KIM_{16-31} (KIM_{16-31m}) and a corresponding T116C mutation (at the end of helix αD) in ERK2 led to a high-resolution structure in which an intermolecular disulfide bridge was observed between the two newly introduced residues.[8] In p38α, this same region hosts a well-defined hydrophobic binding pocket that, in cocrystals with D-motif peptides derived from either a substrate MEF2A or an activator MKK3b, docks the side chain belonging to the ϕ_B residue.[10] In fact, these structures exhibit an overall register shift of the entire consensus motif, with the ϕ_A side chain occupying the pocket normally recognized by ϕ_B in the complexes involving ERK2. However, our previous NMR results indicate that this is not the case when p38α binds KIM_{15-31}, with ϕ_A and ϕ_B recognizing the homologous ERK2 pockets and V31′ entering the cavity occupied by ϕ_B in the p38α−peptide cocrystals.[12] It should be noted that this particular pocket in ERK2 is partially occluded by the T108 (perturbed) side chain, making it less able to host bulky side chains from the ligands. A direct comparison of the perturbations induced by KIM_{15-31} on ERK2 and p38α does in fact show a very similar pattern of perturbations at the Φ_{hyd} subsite (Figure S2 of the Supporting Information). The extensive perturbations seen for loop 8, loop 11, αD, and αE hosting the critical residues of the Φ_{hyd} subsite indicate its full engagement for both kinases. We may therefore conclude that, at saturating concentrations, the overall interaction topology of KIM_{15-31} with the DRS of both kinases is conserved in solution. A closer comparison of the spectral perturbations on a per-residue basis does nevertheless reveal differences (Figure S2). On the ERK2 Φ_{hyd} subsite, KIM_{15-31} causes the largest changes in helix αE and loop 11 (the binding pocket hosting the ϕ_A, i.e., L27′ side chain), while perturbations on helix αD (hosting the ϕ_B, i.e., L29′, and V31′ side chains) are less prominent; spectral perturbations are more uniform on the p38α Φ_{hyd} subsite. Differences between the two kinases can also be seen at their Φ_{chg} subsites. For instance, in p38α, E81, located on loop 4, is perturbed while the corresponding ERK2 residue E79 is not (however, flanking H78 and N80 are perturbed). On the other hand, KIM_{15-31} induces more significant changes on loop 16 in ERK2 than in p38α. ERK2 residues E312, S318, I322, and A323 are perturbed, but the corresponding positions on p38α are not. Also, perturbations on helix αI are seen for only ERK2 (V302, E303, and Q304). It has been suggested that some of these ERK2 residues (E303 and S318) are part of an ERK2-specific network of hydrogen bonds and electrostatic interactions that are remodeled by ligand binding.[8] Thus, our data indicate that while KIM_{15-31} engages the Φ_{chg} subsite in both proteins, the subsequent reorganization of the electrostatics in this region is distinct in each case. The comparatively reduced spectral perturbations observed for the p38α Φ_{chg} subsite may indicate that this region is less involved in KIM_{15-31} binding than ERK2, a possible reason for the difference in affinity as measured using ITC (Table S3 of the Supporting Information).

A more substantial difference between the two kinases is observed upon binding of HePTP (Figure 2 and Figure S3 of the Supporting Information). ITC measurements indicate that HePTP binds both kinases with a higher affinity than KIM_{15-31} (Table S3). However, HePTP induces a significantly larger number of spectral perturbations only on p38α when compared with KIM_{15-31}, including a stronger involvement of the Φ_{chg} subsite, as well as a number of distal residues.[12] NMR and ITC data showed that these latter perturbations were the result of binding of the KIS motif on HePTP. In fact, p38α binds the

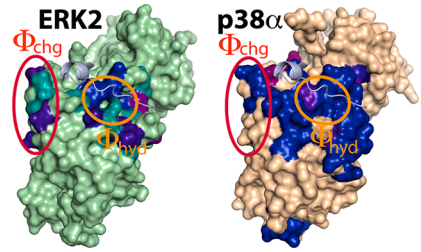

Figure 2. Spectral perturbations induced by HePTP on ERK2 (left) and p38α (right). Color coding as in Figure 1 (ERK2 threshold, −0.052 ppm; p38α residues with perturbations below the threshold colored brown). KIM_{16-31m} peptide (light blue) bound to ERK2 has been modeled onto the p38α surface to aid visualization of the DRS.

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KIMKIS peptide (HePTP residues 15−56 with a C42′S mutation) with an ∼7-fold higher affinity than KIM_{15-31} (Table S3). In contrast, the binding of HePTP to ERK2 produces a set of perturbations not dissimilar to those resulting from KIM_{15-31}, with only a few additional perturbations observed in areas distal to the DRS. This is consistent with an ∼3-fold increase in affinity for HePTP compared with KIM_{15-31} (Table S3). Further, the affinity of the KIMKIS peptide for ERK2 is similar to that of KIM_{15-31} alone [1.8-fold increase (Table S3)]. These observations indicate that the KIS element is less critical for the ERK2−HePTP interaction than for the p38α−HePTP interaction. This observation is in line with an earlier report that demonstrated that various members of the KIM-PTP family (of which HePTP is a member) use their variable KIS sequences to differentially target MAP kinases.[16] With HePTP, the majority of the backbone resonances belonging to the ERK2 DRS move to positions identical to those observed in the presence of KIM_{15-31}, indicating a similar engagement of the D-motif for the two cases. However, a subset of residues located on the Φ_{hyd} subsite, specifically on loops 8 and 11 and helix αD, experience smaller overall spectral perturbations in the presence of the full-length phosphatase (Figure S4 of the Supporting Information). In the case of the resonance corresponding to αD residue T116 (discussed above), the direction of the shift during the titration is the opposite of that for KIM_{15-31}. This suggests a different, perhaps weaker, contact with the C-terminus of the D-motif, and more likely of V31′, in the case of full-length HePTP. For p38α, the additional contacts with KIMKIS compared to those with HePTP that lead to a higher affinity (Table S3) in the former case have been discussed previously.[12] In a SAXS study,[11] we had shown that the catalytic (PTP) domain of HePTP was delocalized in a region below the ERK2 activation loop. The lack of significant spectral perturbations in this region for ERK2 suggests that the catalytic PTP domain of HePTP does not make contact with ERK2, as in the case of p38α.[12]

Thus, D-motif−DRS interactions are essential for binding of HePTP to both p38α and ERK2 in their resting states. However, unlike p38α, in which interactions involving the KIS motif of HePTP significantly enhance binding, for ERK2 these interactions are less important. The catalytic PTP domain of HePTP contributes to the binding of neither p38α nor ERK2. D-Motif−DRS recognition modes for ERK2 and p38α are topologically similar overall and consistent with that depicted by the crystal structure of KIM_{16-31m} bound to ERK2.[8] The specific variations of perturbations for the two kinases likely result from different contributions of the individual portions of the DRS and its constituent subsites in determining the details of their respective binding modes. Interestingly, no other distal perturbations were observed on the N-lobes of ERK2 or p38α upon binding of either KIM_{15-31} or HePTP. A number of crystallographic studies on both these MAP kinases[8,13] showed that the binding of various ligands to the DRS induces a number of distal structural changes that, triggered by conformational rearrangements on loop 4 and loop 16, and through the mediation of helix αC, lead to changes in the Gly-rich loop, the N-terminus, and the activation loop. The activation loop, in particular, was shown to adopt a new well-defined conformation in the case of ERK2[8] and became more flexible for p38α.[13] While resonances belonging to the activation loop of ERK2 in its resting state are currently unassigned, these assignments are complete for p38α. Resonances corresponding to all the other areas mentioned above have been largely identified for both kinases.[9,12,17,18] The lack of spectral perturbations indicates the absence of significant conformational differences between the free and bound states in solution in these regions of either kinase in the presence of the short D-motif peptide or full-length HePTP. Clearly, more work is required to establish if the discrepancy between the solution and crystallographic studies is due to a consistent interaction *in crystallo* or the existence of boundlike states in the solution ensemble.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supporting methods and NMR and ITC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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